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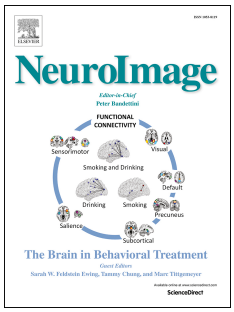


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# The impact of GABAergic drugs on TMS-induced brain oscillations in human motor cortex

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*Highlights*

- The response to TMS of M1 is composed of evoked and induced oscillatory activity
- TMS induced early α-/ß-synchronization and late α-/ß-desynchronization in M1
- GABAAergic vs. GABABergic drugs had opposite effects on early α-synchronization
- GABAAergic and GABABergic drugs enhanced the late ß-desynchronization

## Abstract

Brain responses to transcranial magnetic stimulation (TMS) as measured with electroencephalography (EEG) have so far been assessed either by TMS-evoked EEG potentials (TEPs), mostly reflecting phase-locked neuronal activity, or time-frequency-representations (TFRs), reflecting oscillatory power arising from a mixture of both evoked (i.e., phase-locked) and induced (i.e., non-phase-locked) responses. Single-pulse TMS of the human primary motor cortex induces a specific pattern of oscillatory changes, characterized by an early (30-200 ms after TMS) synchronization in the α- and ß-bands over the stimulated sensorimotor cortex and adjacent lateral frontal cortex, followed by a late (200-400 ms) α- and ß-desynchronization over the stimulated and contralateral sensorimotor cortex. As GABAergic inhibition plays an important role in shaping oscillatory brain activity, we sought here to understand if GABAergic inhibition contributes to these TMS-induced oscillations. We tested single oral doses of alprazolam, diazepam, zolpidem (positive modulators of the GABAA receptor), and baclofen (specific GABAB receptor agonist). Diazepam and zolpidem enhanced, and alprazolam tended to enhance while baclofen decreased the early α-synchronization. Alprazolam and baclofen enhanced the early ß-synchronization. Baclofen enhanced the late α-desynchronization, and alprazolam, diazepam and baclofen enhanced the late ß-desynchronization. The observed GABAergic drug effects on TMS-induced α- and β-band oscillations were not explained by drug-induced changes on corticospinal excitability, muscle response size, or resting-state EEG power. Our results provide first insights into the pharmacological profile of TMS-induced oscillatory responses of motor cortex.

## 1. Introduction

The combination of transcranial magnetic stimulation and electroencephalography (TMS-EEG) is a powerful approach to assess aspects of cortical excitation and inhibition (Chellappa et al., 2016; Chung et al., 2015; Ilmoniemi and Kicic, 2010; Ziemann, 2011). TMS-evoked EEG potentials (TEPs) represent the averaged time-locked brain response to single-pulse TMS in the time domain, which likely involves both excitatory and inhibitory neuronal processes (Rogasch and Fitzgerald, 2013). So far, the most direct evidence for the role of inhibition in the generation of TEPs comes from pharmacological studies that assessed the impact of central nervous system (CNS) active drugs that modulate GABAergic neurotransmission (Darmani et al., 2016; Premoli et al., 2014a; Premoli et al., 2014b). However, pharmacological TMS studies have so far only considered the impact of TMS in the time domain, while also the frequency domain provides relevant and complementary information about the brain response to TMS (Herring et al., 2015; Rosanova et al., 2009).

Calculating the time-frequency representation (TFR) of the oscillatory EEG response to TMS allows to simultaneously assess power levels in a variety of frequencies in a time resolved manner. Importantly, oscillatory responses to TMS can either be evoked (i.e. phase-locked) or induced (i.e. non-phase-locked) by the stimulation, and each single trial can reflect a combination of both (i.e., *mixed* response) (Herrmann et al., 2014; Pellicciari et al., 2017). Evoked responses may either result from an additive neuronal response elicited by the stimulation or a phase-reset of ongoing (*spontaneous*) oscillations (David et al., 2006). Evoked oscillations can be calculated on the average of single-trials (i.e., TEP), as most of the non-phase-locked

activity is cancelled out by averaging in the time domain. In contrast, induced responses result from the stimulus-related modulation of the amplitude but not the phase of an ongoing oscillation, and they are generally estimated by means of event-related synchronization and desynchronization (Pfurtscheller and Aranibar, 1977). Induced oscillations are detected when TFRs are estimated on the single-trial level and averaged subsequently, e.g. when calculating the so-called *event-related spectral perturbation* (ERSP) index (Makeig, 1993). However, ERSP provides a mixed response as this procedure is equally sensitive for induced and evoked oscillations and cannot discriminate the two oscillatory response types (Herrmann et al., 2014; Pellicciari et al., 2017). Previous TMS-EEG studies have either focused on TMS-evoked responses (Bonato et al., 2006; Farzan et al., 2013; Garcia et al., 2011; Herring et al., 2015; Paus et al., 2001) or on mixed responses (Ferrarelli et al., 2008; Ferrarelli et al., 2012; Johnson et al., 2012; Rosanova et al., 2009). For TMS of the hand area of the primary motor cortex ($M1_{HAND}$), mixed oscillatory responses in the α- and β-band have been reported to transiently emerge in proximity of the stimulated site (Brignani et al., 2008; Fuggetta et al., 2005; Paus et al., 2001; Veniero et al., 2011). However, a systematic study of TMS-induced oscillations and their underlying neuronal mechanisms is still lacking.

To further explore TMS-related oscillations as novel indicators of cortical processes we sought to disentangle TMS-induced from the contribution of TMS-evoked oscillations and analyze their GABAergic pharmacological profile. For this purpose, we reanalyzed the data from two previously published experiments (Premoli et al., 2014a), in which the respective roles of different α-subunit-containing GABAARs (Experiment 1)

and GABABRs (Experiment 2) was investigated, as they underlie different physiological (Mohler et al., 2002) and pathological (Cossette et al., 2002) functions. In Experiment 1, we investigated the effects of *alprazolam*, a classical benzodiazepine and positive modulator of α1-, α2-, α3- and α5-containing GABA type A receptors (GABAAR), and *zolpidem*, a short-acting non-benzodiazepine hypnotic drug, which mainly binds to the α1-containing GABAAR. In Experiment 2, we tested the effects of *diazepam*, another classical benzodiazepine binding to α1-, α2-, α3- and α5-containing GABAARs, and *baclofen*, a specific GABAB receptor (GABABR) agonist.

Understanding the neuropharmacological basis of TMS-induced oscillations is of fundamental importance as this knowledge may lead to new markers of excitatory and inhibitory processes in the human brain, which could be used for detecting abnormal excitability in brain disorders.

## 2. Methods and Materials

### *2.1 Subjects*

Twenty-two (Experiment 1, mean age: 25.0 ± 2.5 years, range: 21–32 years) and 19 male healthy subjects (Experiment 2, mean age: 26.4 ± 3.5 years; range: 22–32 years) gave written informed consent before enrolment in this study. Here, the data presented are from 16 and 15 subjects, respectively for Experiment 1 and 2, as several subjects had to be excluded due to data quality issues (see below). The TMS-evoked EEG potential (TEP) analyses of this sample have already been published (Premoli et al.,

2014a) and is outside the scope of this paper. Female participants were not recruited because of menstrual cycle-related effects on cortical excitability, which can be a potential confound in TMS studies (Smith et al., 1999). Subjects were tested for right-handedness following the Edinburgh Handedness Inventory (laterality score ≥75%) (Oldfield, 1971). All participants were screened for contraindications to TMS during a physical and neurological examination (Rossi et al., 2011). Exclusion criteria encompassed the presence of a history of neurological or psychiatric disease, use of CNS active drugs, abuse of any drugs (including nicotine and alcohol) as well as contraindications to the study medications. Experiments were approved by the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte), and by the local Ethics Committees of the Medical Faculty of Goethe-University Frankfurt (Experiment 1) and the Medical Faculty of Eberhard-Karls-University Tübingen (Experiment 2).

*2.2 Experimental design*

We performed two double-blind, randomized, crossover design studies to investigate the impact of GABAAR (Experiment 1) and GABABR (Experiment 2) mediated inhibitory neurotransmission on TMS-induced oscillations. In Experiment 1 we tested the acute effects of a single oral dose of alprazolam (1 mg, Alprazolam ratiopharm, ratiopharm), zolpidem (10 mg, Zolpidem-ratiopharm, ratiopharm), or placebo (P-Tabletten Lichtenstein). In Experiment 2 we tested a single oral dose of baclofen (50 mg Lioresal, Novartis Pharma), diazepam (20 mg Diazepam-ratiopharm, ratiopharm), or placebo (P-Tabletten Lichtenstein).

In both studies, subjects participated in three experimental sessions, randomized in order and separated by one week to avoid drug carryover effects. Both resting state EEG (3 min with eyes open) and TMS-EEG measurements were performed immediately before and 90 min after drug intake (**Figure 1**). In Experiment 1 only 13 subjects underwent resting state EEG measurements. Drug dosages and post-drug measurement time were chosen according to the pharmacokinetic properties of the study drugs (Greenblatt and Wright, 1993; Salva and Costa, 1995; Shader et al., 1984), as well as to our previous TMS-electromyography (TMS-EMG) studies that have demonstrated significant modulation of motor cortical excitability (Ilic et al., 2002; McDonnell et al., 2006; Müller-Dahlhaus et al., 2008).

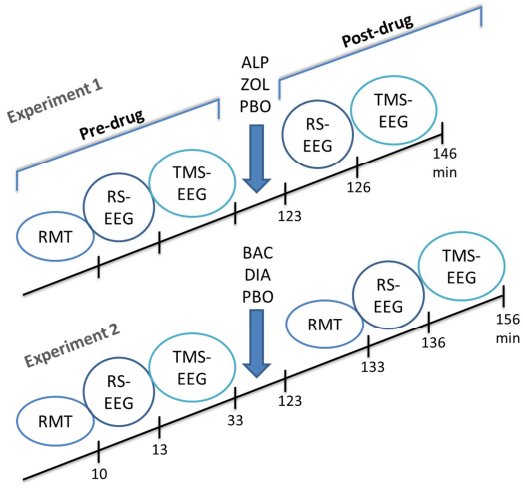


**Figure 1. Timeline of experiments:** Resting Motor Threshold (RMT), 3 min of resting state EEG (RS-EEG) and TMS-EEG measurements were performed before (pre-drug) and 90 minutes after (post-drug) the intake of alprazolam (ALP), zolpidem (ZOL) or placebo (PBO) in Experiment 1, and baclofen (BAC), diazepam (DIA) and PBO in Experiment 2. RMT was not registered post-drug in Experiment 1.

### *2.3 TMS*

A figure-of-eight coil (external diameter of each wing, 90 mm) connected to a Magstim 200$^2$ magnetic stimulator (The Magstim Company Ltd., Whitland, UK) with a

monophasic current flow was used to deliver single-pulse TMS to the left $M1_{HAND}$. We targeted the representation of the right *abductor pollicis brevis* muscle (APB), which was determined as the site where TMS at a slightly suprathreshold intensity consistently produced the largest motor evoked potentials (MEPs) (Rossini et al., 2015). MEP recordings were obtained from surface EMG, using Ag-AgCl cup electrodes in a belly–tendon montage. The EMG raw signal was amplified and bandpass filtered (20 Hz to 2 kHz; D360 amplifier, Digitimer) and digitized at an A/D rate of 10 kHz (CED Micro 1401; Cambridge Electronic Design). The coil was placed tangential to the scalp with the handle pointing backwards and 45° away from the mid line, inducing a posterolateral to anteromedial current in the brain. This is the optimal orientation to transsynaptically activate the corticospinal system via horizontal corticocortical connections (Di Lazzaro et al., 2008). Resting motor threshold (RMT) was determined to nearest 1% of maximum stimulator output, following the relative frequency method (Rossini et al., 2015), that is the minimum intensity necessary to elicit an MEP of ≥ 50 µV in peak-to-peak amplitude in at least 5 of 10 subsequent trials. A number of 150 TMS pulses (Experiment 1) and 125 TMS pulses (Experiment 2) each were applied over the left M1 APB hotspot at an intensity of 100% RMT. In Experiment 1, RMT was tested pre-drug only, and stimulation intensity was kept constant throughout all measurements, whereas in Experiment 2, RMT was tested pre- and post-drug to assess possible drug-induced changes in RMT (**Figure 1**) and re-adjust stimulation intensity to keep it constant at relative to 100% RMT. Per definition, this stimulation intensity elicited MEPs ≥ 50 µV in approximately half of the trials. Only for Experiment 2, EMG was co-registered also during the TMS-EEG measurements. The position of the APB hotspot was marked with

a felt tip pen on the EEG cap to ensure constant coil placement throughout an experimental session. Further, coil position and orientation relative to the marked position were carefully monitored by the experimenter throughout stimulation and corrected if necessary (i.e., if the participant moved). Importantly, any lack of precision (relative to the use of a neuronavigation system) would have resulted only in increased unsystematic error variance (and potentially false negatives), but the double-blind design ensured that no systematic bias (and thus no false positives) could be introduced.

*2.4 High-density EEG recordings during TMS*

A 64-channel electrode cap (BrainCap-Fast'n Easy 64Ch, Brain Products) connected to TMS-compatible EEG amplifiers (BrainAmp DC, BrainProducts) was used to record brain oscillations, hardware-filtered between 0.016 and 1000 Hz and digitized with a 5 kHz sampling rate. Vertical electrooculogram was recorded with 2 additional electrodes to measure eye movements and blinks. Impedances of all electrodes were kept < 5 kΩ. During TMS-EEG recordings, subjects were seated on a comfortable reclining chair and asked to stay awake with eyes open. Masking white noise was played through earphones to avoid contamination of the EEG signal by auditory potentials evoked by the click of the discharging TMS coil (Casarotto et al., 2010; Massimini et al., 2005). The noise intensity was adjusted individually in each experimental session, until the participant reported not being able anymore to hear the TMS-click. While residual input via bone conduction cannot be completely excluded, based on their topography our results cannot be explained by auditory evoked potentials. During EEG co-registration, we applied 2 blocks of 125 TMS pulses each pre- and post-drug over the left $M1_{HAND}$

APB hotspot. The inter-stimulus interval between TMS pulses was on average 5 s with a random inter-trial interval variation of 25% to reduce trial anticipation.

### *2.5 TMS-EEG data analysis*

This study is based on data from our two previously published experiments with an original dataset of 22 (Experiment 1) and 19 (Experiment 2) subjects (Premoli et al., 2014a). In this work, data from 2 subjects in Experiment 1 and 4 subjects in Experiment 2 had to be excluded from final analysis because of excessive artifact contamination of the EEG traces. In addition, in Experiment 1, data from other 5 subjects had to be excluded as they were particularly affected by Magstim $200^2$ capacitors recharge artifact. Note that analyses, including artifact removal, trial rejection, and subject exclusion were performed completely blind towards experimental condition.

TMS-EEG data were analyzed by using MATLAB® (Mathworks Ltd, USA, R2012b) (The Mathworks Inc.), EEGLAB (Delorme and Makeig, 2004) and FieldTrip toolboxes (Oostenveld et al., 2011). After visual inspection, trials affected by prominent artifacts (i.e. eye and muscle movement) were removed, and bad channels were deleted and spatially interpolated. On average, 80 trials were retained for analysis per data set. A linear interpolation was applied between -2 and 6 ms relative to the TMS pulse to remove the initial TMS artifact caused by the step-response of the amplifier. In Experiment 2 an additional linear interpolation (between -102 and -94 ms) was used to remove a TMS-recharge artifact at -100ms. This technical artifact was related to the effectively omitted conditioning pulse (i.e. with intensity of 0 % MSO) from the paired-pulse TMS trials that was part of the same protocol (Premoli et al., 2014b). Data were

then linearly detrended, band-pass (1-45 Hz) and band-stop filtered (49-51 Hz), segmented (-600 to 600 ms), down-sampled to 625 Hz, and referenced to the common average of all electrodes. Independent Component analysis (ICA) was then applied to remove remaining TMS-related artifacts (i.e., the cranial muscle response, the recharging of capacitors, and related exponential decay artifacts (Herring et al., 2015; Korhonen et al., 2011; Rogasch et al., 2016)), as well as further muscle and ocular activity. Artifact components were removed if their spatio-temporal profile indicated the activation of temporal muscles by a characteristic sinusoidal waveform peaking around 5 and 15 ms post-TMS (with opposite sign) over frontotemporal sites close to the temporal muscle (Mutanen et al., 2013). Due to excessive artifact contamination, data from six and four subjects had to be excluded from Experiment 1 and 2, resulting in 16 and 15 subjects, respectively. Note that analyses, including artifact removal, trial rejection, and subject exclusion were performed completely blind towards experimental condition.

Time-frequency representations (TFRs) of TMS-related oscillatory power changes were calculated, separately for each pre- and post-drug condition, by means of a Hanning taper windowed FFT with frequency-dependent window length (width: 3.5 cycles per time window, time steps: 10 ms, frequency steps: 1 Hz from 8 to 30 Hz) (Delorme and Makeig, 2004) (**Figure 2B**). We divided neuronal response components into those *evoked* (i.e. phase-locked) vs. *induced* (i.e. non-phase-locked) by TMS (**Figure 2A**, (Cohen and Donner, 2013; Donner and Siegel, 2011; Herrmann et al., 2014; Pellicciari et al., 2017)). The TFR of the *induced* response was then isolated by subtracting the individual time-domain average from each trial before calculating the

TFRs of the single trials (as done in Cohen and Donner, 2013, but note that we used a sliding window FFT instead of complex Morlet wavelets to calculate the TFRs; **Figure 2D**) instead of subtracting the average TFR of the evoked response form the average TFR of the total response (as suggested in Herrmann et al., 2014; Pellicciari et al., 2017). The former approach was preferred since we performed single-trial normalization by z-transforming the TFR of each trial for each frequency, which the latter approach would not allow. The z-transformation was based on the respective mean and standard deviation derived from the full trial length. This was followed by an absolute baseline correction for each trial by subtracting the average of the -400 to -200 ms period (this artifact-free baseline window was chosen because the presence of a technical artifact at -100ms did not allow a later window; see above) for each frequency to ensure z-values represent a change from pre-TMS baseline. Subsequently, TFRs were averaged across trials per experimental condition. This procedure resulted in an *event related spectral perturbation (ERSP)* measure that is robustly normalized based on the single trial level (Grandchamp and Delorme, 2011), however, with the contribution of the TMS-evoked response removed at the single-trial level. Finally, TFRs were cropped to the time of interest (-400 to 400 ms), removing time-frequency bins at the trial edges for which no values could be computed. Values were averaged across frequency bins to calculate the power within the α (8-12 Hz) and β (13-30 Hz) frequency bands, which represent the dominating oscillations of the sensorimotor cortex (Engel and Fries, 2010; Jensen et al., 2005; Neuper et al., 2005). While this approach for disentangling TMS-induced from TMS-evoked oscillations is naturally imperfect (slight trial-by-trial variations in the latency and amplitude of the TEP waveform are not captured and result in small

subtraction errors, which may be misinterpreted as induced power) it is to the best of our knowledge currently the best method available. In this particular study, the specific pattern of early synchronization and late desynchronization is not observed in TMS-evoked responses and is thus highly unlikely to reflect mere residuals of evoked activity (**Figure 2**).

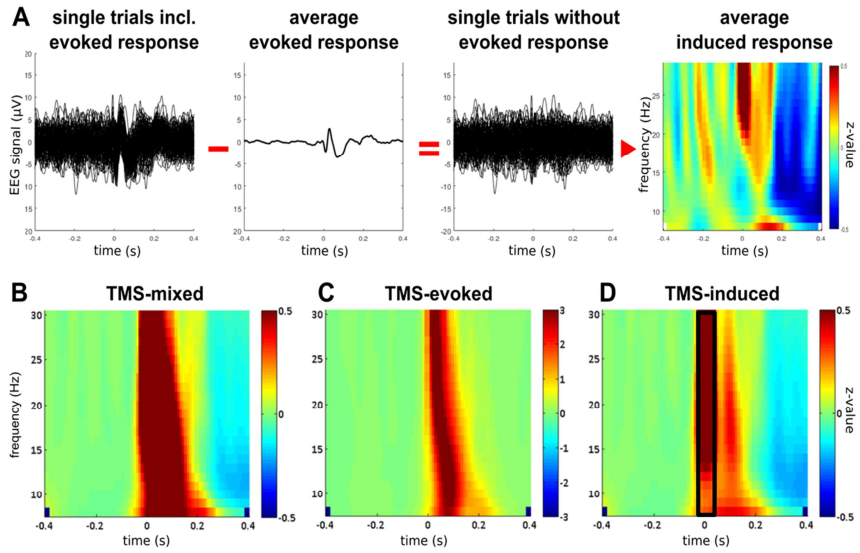


**Figure 2. Disentangling TMS-induced and TMS-evoked oscillations.** (A) Illustration of the method used to disentangle induced from evoked oscillatory responses for a single TMS-EEG session of a representative subject. The average time-locked evoked response is subtracted from every single trial before calculating the TFR, thus capturing the non-phase-locked (i.e. induced) oscillatory responses only (see Methods for details). (B) Time-frequency representation (TFR) of the *mixed* TMS-related power change, shown for conceptual reasons only (obtained by calculating TFRs on the single-trial level *without* previous removal of the evoked response). (C) TMS-evoked oscillations, shown for conceptual reasons only (obtained by calculating the TFR of the individual average TEP, i.e. after averaging the time-locked signal across trials in the time domain). (D) TMS-induced oscillations (obtained by calculating the TFR at single-trial level *after* removal of the evoked component, see Methods section for details). Results are shown for electrode C3 (approximately overlying the stimulated left $M1_{HAND}$) and correspond to the grand-average across subjects and across the three pre-drug conditions of Experiment 1. The black box in proximity of 0 highlights the residual of the TMS artifacts and corresponds to the time window which was not included in the analysis. Note that the common, symmetrical color scaling for all subpanels facilitates visibility of the late desynchronization but causes a saturation for synchronization values

leading to an apparent fusion (particularly for mixed responses in B) of the broadband power increase during the residual TMS-related artifact (approx. -30 to 30 ms) and the subsequent oscillatory synchronization in alpha and beta bands (approx. 30- 200 ms; TOI1). Also note the clear demarcation line with z-values close to zero between these two areas for the TMS-induced oscillations (D) under investigation.

### *2.6 TMS-EMG data analysis*

For Experiment 2, EMG had been co-registered during TMS-EEG measurements and MEP peak-to-peak amplitudes were evaluated for each trial. MEP amplitudes were calculated as difference between the maximum and minimum of the EMG signal as extracted from an individualized search window to account for differences in MEP latency. The individual search window was defined based on the average TMS-locked EMG waveform (but always within the limits of 0.015 to 0.045 s post-TMS). MEP data was only available for both pre- and post-drug measurements for 12 subjects (baclofen) and 13 subjects (diazepam and placebo). Average MEP amplitudes were compared between pre- and post-drug measurements using paired-sample t-tests. Single-trial MEP amplitudes were further used to split the above described analysis of TMS-induced oscillatory power into subthreshold (< 0.05 mV) and suprathreshold (≥ 0.05 mV) trials.

### *2.7 Resting state EEG data analysis*

GABAergic drugs, such as diazepam (Jensen et al., 2005; Saletu et al., 1987), alprazolam (Kaplan et al., 1998), and zolpidem (de Haas et al., 2010) are known to also affect spontaneous oscillations at rest. However, to the best of our knowledge the effect of baclofen has not yet been tested with quantitative EEG in awake human subjects. To

investigate the relationship between drug-induced changes of TMS-induced and spontaneous oscillations, 3 min segments of eyes open resting state EEG data were analyzed as well. Data were initially divided into non-overlapping 2 s time-windows. A Morlet-wavelet convolution (width of 5 cycles, in time steps of 10 ms and frequency steps of 1 Hz from 8 to 45 Hz) was used to analyze the power spectra of the resting state EEG signal before and after drug intake in the α (8-12 Hz) and β (13-30 Hz) frequency bands. We performed a correlational analysis to explore a possible relation of drug-induced changes in TMS-induced and spontaneous resting state EEG oscillations. To pursue this, we extracted the power values for each spectral band, for each subject, before and after drug administration in those channels that showed a drug-induced change in both TMS-induced and spontaneous power (see Results). Those channels were selected to test whether changes in spontaneous oscillations may affect changes in TMS-induced oscillations. Spearman correlation analyses were run between the TMS-induced power change (power post-drug minus power pre-drug) and the resting state EEG power modulation (power post-drug minus power pre-drug) in the different drug conditions.

### *2.8 Statistics*

Significant differences in TMS-induced α-band (8-12 Hz) and β-band (13-30 Hz) power changes from baseline (pre-TMS) were assessed using non-parametric permutation tests based on one-sample t-statistics (Maris and Oostenveld, 2007) comparing the z-normalized TFRs against zero, as the pre-TMS baseline is zero on average by definition (due to the above described baseline subtraction after full trial z-normalization). The analysis was performed in two fixed time windows of interest

(TOIs), an early (30-200 ms; TOI1) and a late (200-400 ms; TOI2) window. TOIs were chosen on the basis of the grand average of the pooled pre-drug measurements to track power changes over time, and TOIs were kept identical for each statistical comparison (**Figure 2D**) (Grent-'t-Jong et al., 2016). Note that TOI1 is close to spurious power increases due to residual TMS-artifacts (approx. -30 to 30 ms), but that (i) a clear demarcation line can be seen between the two areas for the TMS-induced oscillations under investigation (cf. Figure 2D, Figure 3, and Figure 5), and that (ii) no systematic confounds can arise from any residual TMS-related artifacts (as they are independent of the pharmacological interventions).

To analyze drug modulation on TMS-induced α- and β-bands oscillatory power, multiple non-parametric permutation tests based on dependent-sample t-statistics (Maris and Oostenveld, 2007) were run between the different conditions (post- vs. pre-drug), separately for each drug condition and TOIs. To correct for multiple comparisons due to the large number of electrodes and time points within TOIs, we additionally conducted cluster based permutation tests (Maris and Oostenveld, 2007) as implemented in FieldTrip (http://fieldtrip.fcdonders.nl). Thus, a non-parametric permutation test (Monte-Carlo method based on paired t-statistics) comparing the post-drug versus pre-drug condition was performed for each electrode at each time bin within the two different TOIs while averaging over the frequency bins of the spectral band of interest. Then, t-values exceeding an *a priori* threshold of $p < 0.05$ were clustered based on adjacent time bins and neighboring electrodes. Cluster-level statistics were calculated by taking the sum of the t-values within each cluster. The statistical comparisons were performed with respect to the maximum values of summed t-values. By means of a permutation

test (i.e., randomizing data across post-drug and pre-drug conditions and rerunning the statistical test 1500 times), we obtained a reference distribution of the maximum of summed cluster t-values to evaluate the statistic of the actual data. Clusters in the original dataset were considered to be significant at $p < 0.05$ if < 5% of the permutations used to construct the reference distribution yielded a maximum cluster-level statistic larger than the cluster-level value observed in the original data.

Finally, the cluster-based permutation approach was also used to evaluate drug-induced power changes in spontaneous α- and β-band oscillations in the resting state EEG (i.e., in the absence of TMS). Multiple dependent t-tests were run between the different conditions (post- vs. pre-drug), separately for each drug condition.

## 3. Results

### *3.1 Characterization of TMS-induced oscillations before drug intake*

Compared to pre-TMS baseline, time-frequency representation of TMS-induced oscillations revealed a significant power increase (i.e. synchronization) in the β-band (13-30 Hz) in TOI1 ($p < 0.001$, from 30 to 200 ms in both Experiment 1 and 2) and a significant β-band power decrease (i.e. desynchronization) encompassing bilateral sensorimotor cortices in TOI2 ($p < 0.05$, from 200 to 400 ms in both Experiments; **Figure 3A-B**). For the α-band (8-12 Hz), only the early synchronization in TOI1 was significant ($p = 0.001$ for both Experiments) but not the late desynchronization ($p > 0.05$; **Figure 3A-B**); but there were significant increases in α-band power in TOI2 for frontal and posterior clusters ($p = 0.003$ and $p = 0.03$, respectively). The topographic distribution of induced power changes for α- and β-bands, shown in **Figure 3C**,

suggests maximal effects over the stimulated left M1 in both TOIs, with an additional homologue cluster in the contralateral right hemisphere (usually smaller and located slightly more anterior) for the α- and β-power desynchronization in TOI2. Topographical information is only based on sensor-level data, and should thus be interpreted with care.

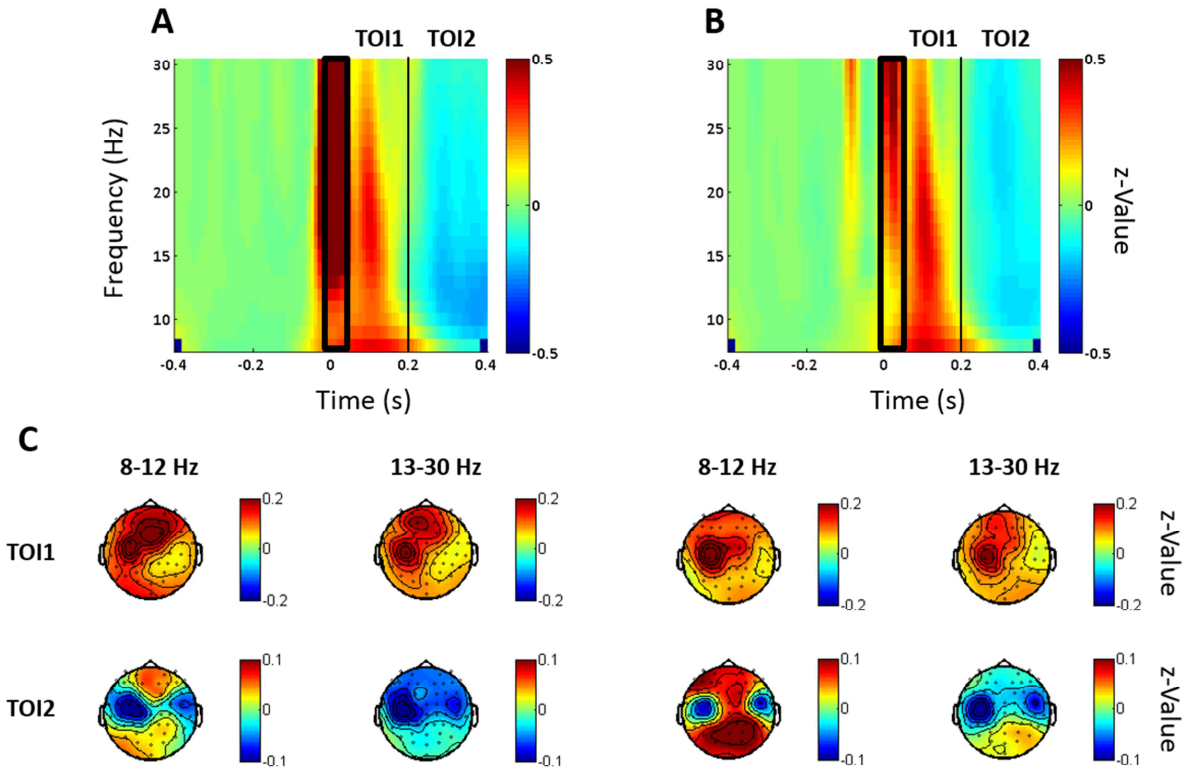


**Figure 3. Characterization of TMS-induced oscillations at pre-drug baseline.** Grand average of the time-frequency representation (TFR) of TMS-induced oscillations (averaged across subjects and the three pre-drug measurements) recorded at the C3 electrode as the best representation of the stimulated left $M1_{HAND}$ in Experiment 1 (A) and Experiment 2 (B). TMS induced β-band (13-30 Hz) synchronization in TOI1 (30-200 ms) and ß-desynchronization in TOI2 (200-400 ms). Topographical distribution of the average power in the respective frequency band at sensor level is shown in (C) for both TOI1 (top row) and TOI2 (bottom row) in each of the analyzed frequency bands. High-frequency noise at -100ms shown in panel B represents residual TMS-recharge artifact after interpolation due to the paired-pulse protocol (note that no actual TMS pulse was applied at -100 ms in the analyzed trials, see Methods section). The black boxes in proximity of 0 sec in A-B highlight the residual of the TMS artifacts and correspond to the time window which was not included in the analysis.

*3.2 Effects of GABAAR mediated inhibition on TMS-induced oscillations: Experiment 1*

Cluster-based permutation analyses revealed no differences in oscillatory power between pre-drug conditions (all $p > 0.05$). Alprazolam resulted in a trend towards increased α-band synchronization in TOI1 ($p = 0.08$), but significantly reduced ß-band synchronization in TOI1 ($p = 0.02$), and increased ß-band desynchronization in TOI2 ($p = 0.04$; **Figure 4A**). Both effects appeared over left fronto-central electrodes. Zolpidem increased the TMS-induced α-band synchronization in TOI1 over channels close to the stimulated left $M1_{HAND}$ ($p = 0.005$; **Figure 4B**). Placebo showed no effect in any of the frequency bands or TOIs (all $p > 0.05$). The comparison of post-drug conditions to placebo confirmed that zolpidem increased α-band synchronization in TOI1 ($p = 0.01$), and that alprazolam decreased ß-band synchronization in TOI1 ($p = 0.002$) and increased ß-band desynchronization in TOI2 ($p = 0.03$).

*3.3 Effects of GABAAR and GABABR mediated inhibition on TMS-induced oscillations: Experiment 2*

Cluster-based permutation analyses showed no differences in oscillatory power between pre-drug conditions ($p > 0.05$). Baclofen significantly reduced TMS-induced oscillations in the ß-band in TOI1 in a widespread area including the stimulated left sensorimotor cortex as well as left frontal and right parieto-occipital regions ($p = 0.003$). This effect was followed during TOI2 by an increase of α- and β-band desynchronization with a roughly comparable topography (both $p = 0.001$; **Figure 4C**). Diazepam increased α-band synchronization in TOI1 at electrodes close the stimulated sensorimotor cortex ($p = 0.04$), and increased β-band desynchronization in TOI2 mainly

in right frontal and centro-parietal sites (p = 0.01; **Figures 4D**). No significant modulation of TMS-induced oscillations was observed in the placebo condition in either TOI (p > 0.05). The comparison of post-drug conditions to placebo confirmed that baclofen decreased ß-band synchronization in TOI1 (p = 0.003), and that diazepam increased ß-band desynchronization in TOI2 (p = 0.04).

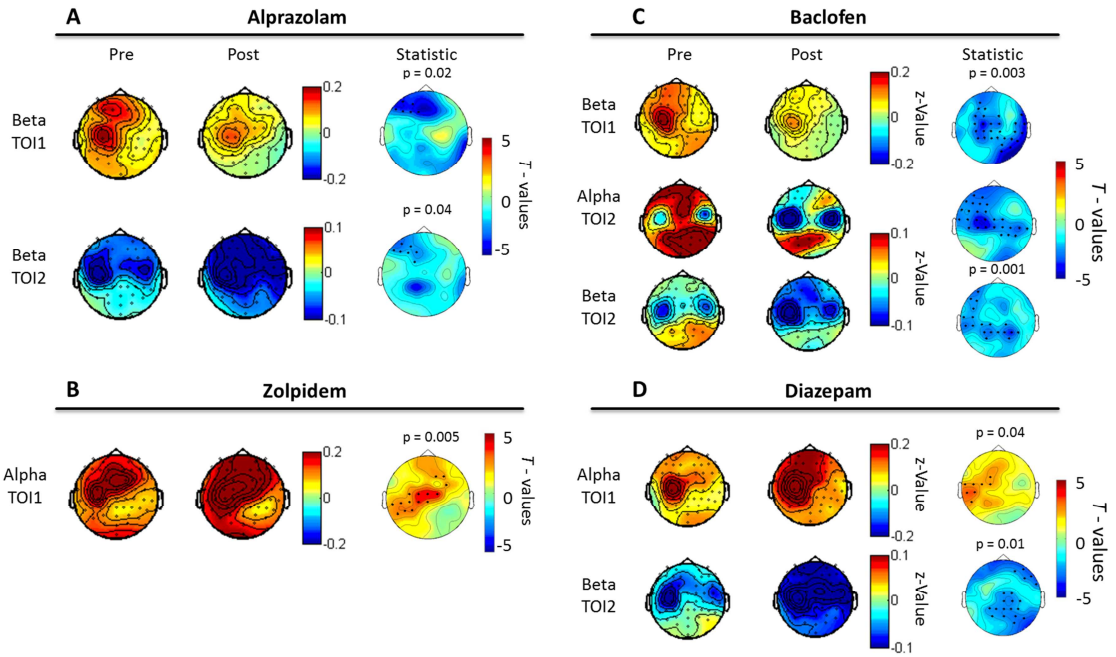


**Figure 4. Topographies of significant drug-induced changes in TMS-induced oscillatory power.** Topographies of drug-induced changes in TMS-induced oscillatory power are shown for alprazolam (A) and zolpidem (B) from Experiment 1 and for baclofen (C) and diazepam (D) from Experiment 2, separately for α- (8-12 Hz) and β-band (13-30 Hz) power and for TOI1 (30-200 ms) and TOI2 (200-400 ms). (A) Alprazolam decreased β-band synchronization in TOI1 and further increased β-band desynchronization in TOI2, both in left frontal regions. (B) Zolpidem only increased α-band synchronization in TOI1 in left centro-parietal and right frontal regions. (C) Baclofen decreased β-band synchronization in TOI1 and increased both α- and β-band desynchronization in TOI2. (D) Diazepam increased TMS-induced α-band synchronization in TOI1 in left central regions and increased β-band desynchronization in right frontal and medial centro-parietal regions. Significant electrodes (p < 0.05, cluster corrected) are denoted with crosses in the t-statistic maps.

### *3.4 Effects of drugs on MEPs*

We aimed to ensure that the observed drug changes in TMS-induced oscillations cannot be explained by concurrent pharmacological changes in either corticospinal excitability or somatosensory re-afferent feedback due to the TMS-evoked muscle twitch, a potential confounding factor (Fecchio et al., 2017; Petrichella et al., 2017). Therefore, we analyzed the average amplitude of MEPs and the proportion of trials with suprathreshold MEPs obtained during the TMS-EEG recordings of Experiment 2 (no EMG data was co-registered during TEP measurements for Experiment 1). Since TMS intensity for TEP recordings was set to 100% RMT, roughly 50% each of trials with subthreshold and suprathreshold MEP were obtained (**Table 1**). RMT increased after the intake of baclofen ($p = 0.028$) and diazepam ($p = 0.004$), but not after placebo ($p > 0.2$). After post-drug re-adjustment of stimulus intensity to 100% RMT, there were no post- vs. pre-drug differences for average MEP amplitude or the proportion of suprathreshold trials in any of the drug conditions (**Table 1**).

### *3.5 Contribution of MEP-related afferent feedback to TMS-induced oscillatory power*

Since somatosensory stimulation alone can induce α- and β-desynchronization (Neuper et al., 2005), and TMS-evoked muscle twitches in the contralateral hand muscles are inevitably associated with somatosensory afferent feedback from muscle spindles reentering the sensorimotor cortex, we tested for Experiment 2 (for which MEP data was available) whether the observed TMS-induced α- and β-band desynchronization can be explained by the presence or absence of suprathreshold MEPs > 0.05 mV

485 (Rossini et al., 2015). According to the stimulation intensity of 100% RMT, per definition
486 roughly half of the trials were supra- and subthreshold trials, respectively (**Table 1**).
487

**Table 1: RMT (% maximum stimulator output, %MSO), MEP amplitudes (mV) and proportion of suprathreshold MEPs (%) for pre- and post-drug conditions of Experiment 2.**

| | pre-drug | | | post-drug | | | t-test (post-pre) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **RMT %MSO** | **Amp (mV)** | **% supra** | **RMT %MSO** | **Amp (mV)** | **% supra** | **RMT** | **Amp** | **% supra** |
| **Baclofen** | 44.07 ± 4.37 | 0.15 ± 0.1 | 50.43 ± 18.16 | 44.87 ± 4.79 | 0.20 ± 0.12 | 58.66 ± 16.28 | $t_{15}$ = 2.45 p = 0.028 | $t_{11}$ = 1.31 p = 0.22 | $t_{11}$ = 1.10 p = 0.29 |
| **Diazepam** | 44.60 ± 5.04 | 0.16 ± 0.11 | 53.19 ± 23.09 | 46.80 ± 5.71 | 0.17 ± 0.11 | 51.58 ± 20.64 | $t_{15}$ = 3.51 p = 0.004 | $t_{12}$ = 0.06 p = 0.95 | $t_{12}$ = -0.15 p = 0.88 |
| **Placebo** | 44.80 ± 4.75 | 0.18 ± 0.10 | 56.62 ± 20.59 | 45.20 ± 4.57 | 0.23 ± 0.16 | 56.65 ± 26.06 | $t_{15}$ = 1.19 p = 0.25 | $t_{12}$ = 1.91 p = 0.08 | $t_{12}$ = 0.18 p = 0.86 |

Mean ± SD as well as t-statistic and p-value are provided for post-pre comparisons per drug condition. Different degrees of freedom result from varying number of available datasets (N = 15 for RMT measurements; N = 12 (baclofen) and N = 13 (diazepam and placebo) for co-registered EMG (MEP and % supra) during TEP measurements.

We thus repeated the above described analyses of TMS-induced oscillations in the α- and β-bands during TOI1 and TOI2, separately for suprathreshold and subthreshold MEP trials, pooled over all pre-drug measurements of Experiment 2 (to gain sufficient trial numbers and thus statistical power). While no differences were observed in either frequency band for the early synchronization (TOI1) between suprathreshold and subthreshold MEPs (all $p > 0.05$), we found indeed stronger β-band desynchronization in TOI2 for trials with suprathreshold compared to subthreshold MEPs ($p = 0.03$), whereas a similar effect for α-band desynchronization remained non-significant ($p > 0.05$) (**Figure 5**). Low trial numbers and thus reduced statistical power after trial splitting did not allow statistical post-pre cluster t-test comparisons divided by sub- and suprathreshold trials and drug condition.

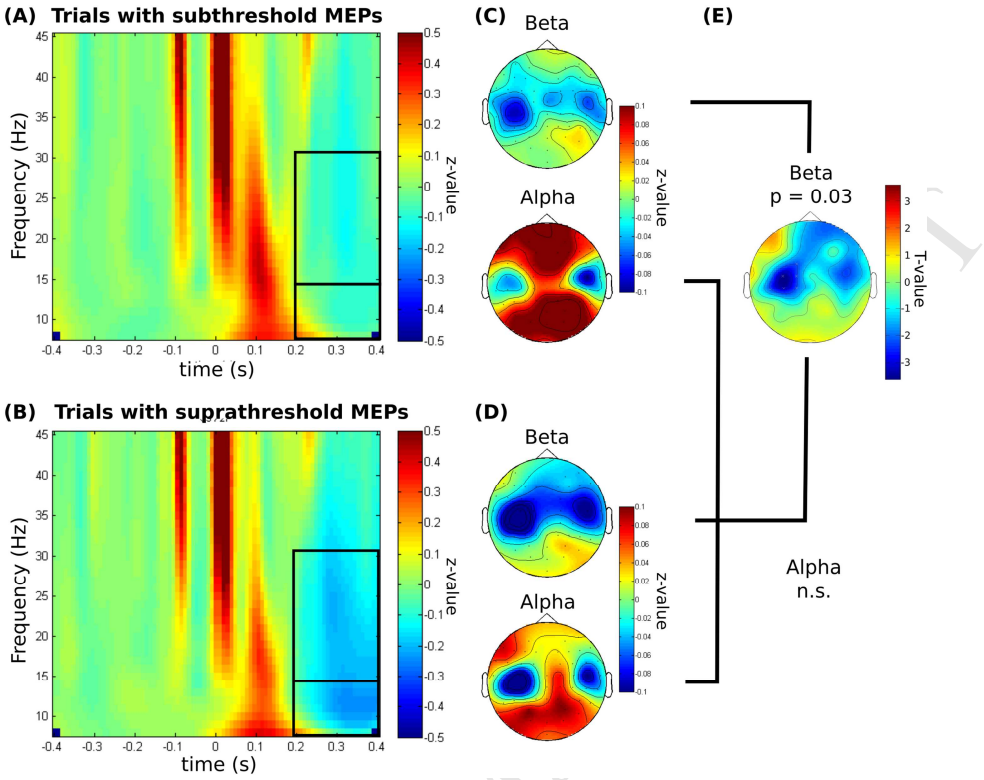


**Figure 5. Comparison of TMS-induced α- and β-desynchronization for trials with supra- vs. subthreshold MEPs at pre-drug baseline.** TFRs suggest that late α- and β-desynchronization in TOI2 was smaller for (A) trials with subthreshold MEPs than for (B) trials with suprathreshold MEPs at electrode C3 (roughly overlying the left sensorimotor cortex). Topographical representations of TMS-induced α- and β-band power show that for both (C) sub- and (D) suprathreshold MEPs, desynchronization was confined to the stimulated left (and to a lesser degree contralateral right) sensorimotor cortex. (E) Direct comparison of supra vs. subthreshold MEP trials using cluster t-statistics, revealed significantly larger β-band desynchronization associated with supra- than subthreshold MEPs over the left sensorimotor cortex, whereas α-band desynchronization showed the same tendency but did not reach significance. Data were pooled over all pre-drug measures of Experiment 2 to gain sufficient statistical power despite trial splitting.

### *3.6 Effects of drugs on resting state EEG*

We finally analyzed the impact of each drug on resting state spontaneous oscillatory activity without TMS events (i.e. resting state EEG). In Experiment 1, alprazolam increased spontaneous β-band power at fronto-central sites (n=13, p = 0.02; **Figure 6**) but had no significant effect on α-band power (p > 0.05), whereas no significant modulations could be observed for zolpidem and placebo (p > 0.05). The lack of effect

by zolpidem could be explained by the fact that we obtained resting state EEG in the eyes open condition, and that its increasing effect on ß-band power has disappeared after 90 min (Patat et al., 1994). In Experiment 2, baclofen significantly enhanced spontaneous α-band power at lateral frontal as well as medial parietal sites ($p < 0.001$), and β-band power at medial frontal sites ($p = 0.04$); diazepam decreased α-band power at a right lateral central cluster ($p = 0.02$) and increased β-band power at medial frontal sites ($p = 0.003$; **Figure 6**), whereas placebo did not induce any significant changes ($p > 0.05$). Importantly, the above described drug effects on TMS-induced oscillatory power are unlikely to be explained by ceiling effects resulting from drug-induced changes of spontaneous brain oscillations. While their topographies partially overlap (both comprising medial and lateral frontal as well as medial parietal sites), and increases in spontaneous EEG power are matched by decreases in TMS-induced synchronization or increases in desynchronization (cf. **Figure 5** to **Figure 4**), there was no significant correlation between the drug-induced changes in spontaneous α- or β-band resting state EEG oscillatory power and the respective drug effects on TMS-induced oscillations (all $p > 0.3$).

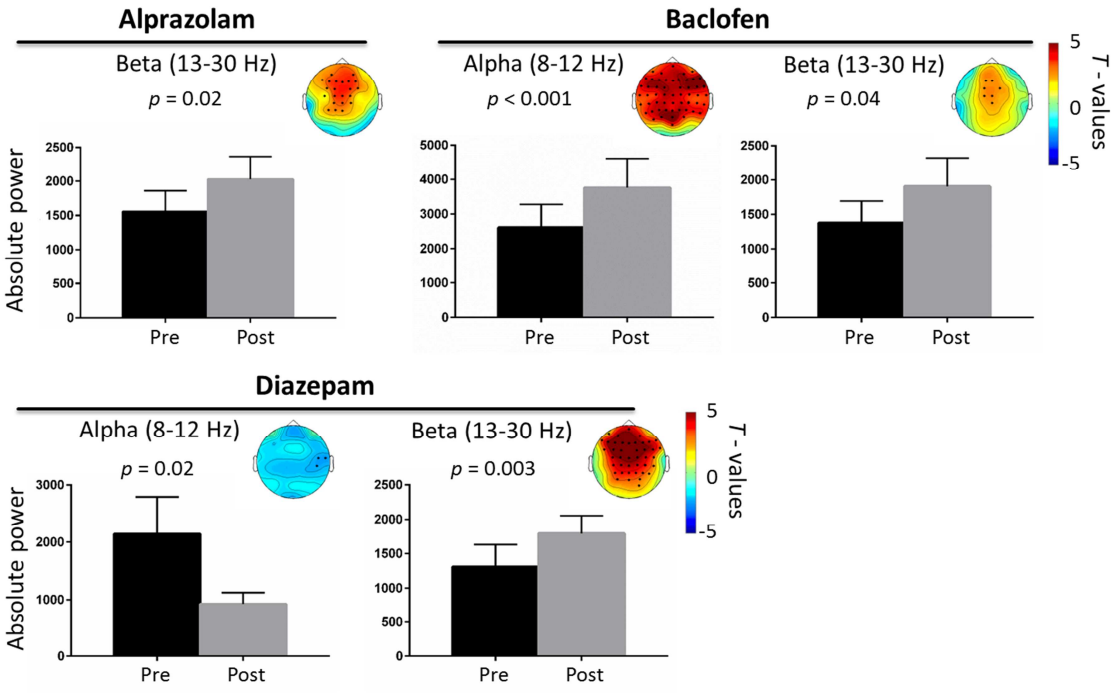


**Figure 6. Effects of drugs on resting state EEG (eyes open):** Bar charts represent absolute EEG power (mean ± SEM) pre (black) and post (grey) drug intake. Each plot shows the grand average across all subjects and the significant channels, which are indicated by black dots in the t-statistic maps. Red represents as increase of power, whereas blue depicts a decrease. For further details, see Material and Methods.

## 4. Discussion

We disentangled for the first time the mixed activity that is usually captured by TMS-related spectral perturbation measures to investigate the specific contribution of induced oscillatory power after removal of the evoked responses (**Figure 2**), the importance of which has recently been highlighted by Pellicciari et al. (2017). TMS induced a specific pattern of oscillatory power in the α- and ß-bands, characterized by an early synchronization over the stimulated sensorimotor and adjacent frontal cortex and a subsequent profound desynchronization over the stimulated and contralateral sensorimotor cortex (**Figure 3**). Pharmacological manipulation (**Figure 4**) suggested

555 that GABAAR mediated inhibition contributes to the early α-band synchronization (30-
556 200 ms after the TMS pulse) in the stimulated sensorimotor cortex, as it was increased
557 exclusively by GABAAR positive modulators (i.e., diazepam and zolpidem, and a trend
558 towards increase by alprazolam). Furthermore, all drugs except zolpidem increased the
559 late ß-band desynchronization (200-400 ms after the TMS pulse) over lateral frontal and
560 medial parietal sites, suggesting an involvement of both GABAAergic and GABABergic
561 processes. Finally, baclofen increased the late α-band desynchronization over the
562 stimulated sensorimotor cortex and its contralateral homologue but also adjacent lateral
563 frontal regions, suggesting the specific contribution of late GABABR mediated inhibition.
564 Importantly, while late α- and β-desynchronization were partially driven by
565 somatosensory re-afferent feedback from the evoked muscle twitch (**Figure 5**), the
566 observed pharmacological modulations of TMS-induced oscillatory activity cannot be
567 attributed to pharmacologically induced changes in corticospinal excitability (**Table 1**),
568 the amount of sensorimotor re-afferent feedback from the muscle twitch (**Figure 5**), or
569 changes in the resting state EEG power in the respective frequency bands (**Figure 6**).

570 *4.1 TMS-induced oscillations in the sensorimotor cortex*

571 We characterized the spatiotemporal profile of oscillations induced by single-pulse TMS
572 of left $M1_{HAND}$. In line with the idea of site-specific 'natural frequencies' (Ferrarelli et al.,
573 2012; Rosanova et al., 2009), EEG responses were dominated by specific dynamics in
574 the α- and β-bands, the prevalent oscillations of the sensorimotor system (Neuper et al.,
575 2005). TMS-induced oscillations showed an initial synchronization over the stimulated
576 sensorimotor and adjacent lateral frontal cortex, followed by a subsequent

desynchronization over the stimulated sensorimotor cortex as well as its contralateral homologue (**Figure 3**). TMS-evoked responses in the α- and β-band have previously been reported (Brignani et al., 2008; Fuggetta et al., 2005; Paus et al., 2001; Van Der Werf and Paus, 2006; Van Der Werf et al., 2006; Veniero et al., 2011), but the non-phase-locked modulation of α- and β-band oscillations has not been described yet. What may be their underlying mechanisms? MEP-informed control analyses, contrasting trials with suprathreshold vs. subthreshold MEPs (with identical stimulation intensity), suggest that the late β-band desynchronization over the stimulated (and contralateral) sensorimotor cortex was partially driven by muscle twitch-related somatosensory re-afferent feedback associated with suprathreshold MEPs (**Figure 5**). Indeed, both movement and sensory stimulation are well known to desynchronize α- and β-band oscillations in the sensorimotor cortex (Neuper et al., 2006; Pfurtscheller, 2001). However, these results do not exclude the possibility that a third factor, for example spontaneous fluctuations of corticospinal excitability, accounted for both the amount of TMS-induced cortical desynchronization and the MEP amplitude. In that case, the observed β-band desynchronization would be a direct consequence of the cortical stimulation, accompanied by corresponding variations in MEP amplitude. To eventually solve this question, one would need to block sensory afference independent of corticospinal excitability or stimulation intensity, e.g., by transient ischemic forearm deafferentation (Ziemann et al., 1998).

Moreover, the early synchronization of the stimulated sensorimotor cortex does not seem to be dependent on the TMS-evoked muscle twitch, as it did not differ between sub- and suprathreshold MEPs (**Figure 5A-B**), and may thus rather reflect the

600 direct transcranial excitation of the oscillation-generating circuits in the motor network.
601 Commonly, β-band synchronization is observed during the so called 'rebound' phase,
602 which follows about 200-600 ms after an initial stimulus- or movement-induced
603 desynchronization and is usually not observed for the α-band (Neuper et al., 2006). In
604 line with our findings, this pattern is typically located in bilateral sensorimotor cortex,
605 with the β-band rebound specifically peaking in M1 contralateral to the movement
606 (Jurkiewicz et al., 2006; Neuper et al., 2006). However, the TMS-induced
607 synchronization preceded the desynchronization and thus cannot be attributed to a
608 rebound phenomenon.

609 Previous TMS-EEG work has described a topographically wide-spread net
610 increase in the α- and β-band oscillatory EEG power within 530 ms after the TMS pulse,
611 scaling with stimulation intensity (Fuggetta et al., 2005). However, early and late
612 intervals were not distinguished, and induced oscillations were not analyzed separately
613 from evoked responses. Therefore, evoked components may have considerably
614 influenced these findings. Our results are in line with the oscillatory pattern observed in
615 the local field potential of the subthalamic nucleus (STN) following TMS of the ipsilateral
616 (and even contralateral) M1, as recorded via intracranial electrodes for deep brain
617 stimulation in patients with Parkinson's disease (Doyle Gaynor et al., 2008). Importantly,
618 TMS at both suprathreshold and subthreshold intensities caused an initial β-band
619 synchronization before 200 ms and a subsequent prolonged desynchronization (200 to
620 600 ms) of STN β-band oscillations, again suggesting that β-band desynchronization is
621 not entirely driven by sensory feedback. But also in that study, the induced and evoked
622 oscillations were not disentangled, and no concurrent scalp EEG was recorded, leaving

the origin of the oscillatory change unresolved. Interestingly, no effect on α-band power was observed in the STN, in line with the idea that TMS-induced α- and β-band oscillations engage separate, though partially overlapping, circuits. While it has been suggested that rolandic α- and β-band oscillations emerge from somatosensory and motor networks, respectively (Salmelin and Hari, 1994), α-band oscillations have also been recorded from layer III of the rat M1, whereas β-band oscillations dominated in layer V (Ronnqvist et al., 2013). In summary, the observed TMS-induced synchronization-desynchronization dynamic of α- and particularly β-band oscillations over sensorimotor cortices is likely to be a direct consequence of the transcranial activation of α- and β-oscillation generating cortico-cortical and cortico-subcortical circuits, while re-afferent feedback from the muscles may further enforce cortical desynchronization.

*4.2 Pharmacological modulation of TMS-induced oscillations*

Inhibitory GABAergic neurotransmission is critical for synchronization of neural activity and generation of network oscillations (Wang, 2010). For instance, both α- and β-band oscillations have been linked to inhibitory function, i.e. the suppression of perceptual input processing via 'pulsed inhibition' (Jensen and Mazaheri, 2010; Klimesch et al., 2007; Mathewson, 2011) and the control of motor output during response inhibition (Picazio et al., 2014; Zhang et al., 2008). Further, the period of decreased motor cortical excitability following electrical median nerve stimulation (Chen et al., 1999) may be related to the stimulation-induced β-band rebound. Accordingly, GABAergic drugs also modulated TMS-induced oscillatory power synchronization at early (30-200 ms) and desynchronization at late (200-400) post-TMS intervals, for both the α- and β-bands

(**Figure 4**). The rather complementary effects of GABAAR and GABABR mediated inhibition suggest that TMS-induced early synchronization and late desynchronization may be dominated by different inhibitory mechanisms. Both broad and α-1 subunit specific GABAAergic drive enhanced the early α-band synchronization. In contrast, the late ß-band desynchronization was under the influence of subunit-unspecific GABAAR and GABABR activity, whilst late α-band desynchronization was exclusively driven by GABABR neurotransmission. It is important to highlight that the two benzodiazepines diazepam and alprazolam showed highly similar effect patters, but not identical with respect to their statistical significance. Two main points have to be considered that may have contributed independently to these slight differences: (i) the two drugs were administered in two separate experiments to independent subject samples, rendering direct between-study comparisons difficult; (ii) the two drugs are highly similar but not identical, and slight differences in the pharmacokinetics may have had an impact. For instance, diazepam is more likely than alprazolam to cause drowsiness, but alprazolam is reported to have more severe withdrawal effects on discontinuation. Finally, alprazolam has a shorter half-life compared to diazepam.

How does the observed GABAergic modulation of TMS-induced oscillatory power relate to the GABAergic impact on spontaneous α- and β-band oscillations at rest and during movement-related modulation? At rest, GABAAR positive modulators consistently increase β-band power (in rolandic and frontal regions), whereas they often reduce α-band power (usually reported for parieto-occipital and not specifically for rolandic cortex). These opposing effects have been demonstrated for all drugs tested in this study, namely diazepam (Jensen et al., 2005; Saletu et al., 1987), alprazolam

(Kaplan et al., 1998; Saletu et al., 1994), and zolpidem (de Haas et al., 2010). For diazepam, we could replicate the power increase and decrease for β- and α-bands, respectively, and we also observed β-band power increase for alprazolam, but contrary to our expectations, no effects for zolpidem (**Figure 6**). The effect of the GABABR agonist baclofen on the resting state EEG had not been investigated before in humans. We showed a strong and topographically widespread drug-induced increase in both spontaneous α- and β-band power (**Figure 6**), to some extent similar to an increase in β- (but not α-) band power shown in DBA/2J mice (Marrosu et al., 2006).

Conversely, during and directly after movement, GABAergic inhibition appears to enhance *movement related β-band desynchronization* (MRBD) but reduce subsequent *post-movement β-band rebound* (PMBR). While bulk GABA concentration in $M1_{HAND}$, as measured by magnetic resonance spectroscopy (MRS), shows a positive relationship with individual PMBR (Gaetz et al., 2011), pharmacological manipulation of its synaptically active portion revealed the opposite. Tiagabine, a GABA reuptake inhibitor and thus receptor unspecific, increased spontaneous β-band power at rest, but enhanced MRBD and reduced PMBR (Muthukumaraswamy et al., 2013), whereas diazepam also enhanced MRBD, but did not affect PMBR (Hall et al., 2011). The authors suggested that MRBD is GABAAR dependent, whereas PMBR may be GABABR dependent. Thus, TMS-induced β-band oscillations seem to be more similar to movement-related than resting β-band oscillations, as both GABAA- and GABABergic inhibition enhanced late β-band desynchronization, and GABABR mediated inhibition also enhanced late α-band desynchronization, while GABAergic effects on spontaneous and TMS-induced oscillations did not correlate. It remains unclear why the

pharmacological enhancement of GABAergic inhibition synchronizes spontaneous but desynchronizes movement-related and TMS-induced β- (and α-) oscillations. However, our results suggest that GABAergic inhibition plays a different role in the resting and activated motor cortex.

Importantly, the observed GABAergic effects on TMS-induced oscillations cannot be explained merely by TMS-evoked muscle twitches or the associated re-afferent somatosensory feedback. Firstly, drug-induced changes in TMS-induced oscillations mainly occurred over medial and lateral frontal as well as medial parietal sites, for which no somatosensory feedback-related activation was observed (**Figure 4** vs. **Figure 5**). Secondly, diazepam and baclofen did alter TMS-induced α- and β-band oscillatory power in Experiment 2, although TMS-evoked muscle responses, and thus somatosensory afferent feedback, were unchanged, because stimulation intensity was kept constant at 100% RMT (**Table 1**).

The question of whether or not to re-adjust stimulation intensity after a pharmacological intervention based on a certain excitability estimate (here the RMT) is difficult to answer, and there are advantages and disadvantages of both options. In fact, this dilemma holds true for any kind of intervention that modulates the excitability of certain neuron populations in the target region while using a network response (such as TMS-evoked or -induced EEG responses) as a readout. In the case of the motor cortex, readjustment of stimulation intensity to compensate for drug-induced RMT changes ensures comparable excitation of the corticospinal system and thus keeps TMS-evoked muscle responses and related sensory feedback constant. However, there are two important unknowns. Firstly, it is unclear the excitability change of which specific neuron

population gave rise to the RMT change in the first place (corticospinal motor neurons in layer IV, connected pyramidal neurons in layer II-III, interneurons, or even spinal motor neurons). And secondly, it is unknown which specific neuron populations (and even which brain regions) give rise to the TMS-evoked/-induced EEG response. Thus, if RMT reflects the excitability of cortical motor neurons, adjustment will ensure that those neurons will be effectively excited to the same degree before and after drug intake, and a changed EEG response is attributable to changed excitability of other connected regions or changed functional connectivity within the same network. Conversely, no adjustment would result in different excitation and consequently different output levels of the cortical motor neurons, ranging from no output at all (if stimulation became subthreshold) to a strong enhancement of the output to connected regions, limiting the interpretability of changed network responses. However, different stimulation intensities may also affect different neuron populations, and for other co-stimulated neuron populations, unaffected by the RMT change but involved in the network response, adjustment would result in different levels of effective excitation and thus different EEG responses (Casarotto et al., 2010). Thus, re-adjustment to RMT can be both a necessity and a potential confound when investigating changes in TMS-evoked/induced oscillatory activity after drug intake (or any other intervention). While on the expense of consistency and direct comparability between the two experiments, we were also fortunate having employed both approaches in Experiment 1 and 2, respectively, using drugs with highly comparable GABAergic effects (i.e., alprazolam and diazepam) and finding largely comparable effects for both (**Figures 4A, D**). Future studies, should thus evaluate post-drug (or generally post-interventional) measures with both adjusted and

unadjusted intensities to overcome these limitations. For target sites outside M1 (lacking an index such as RMT, maybe requiring electric field estimates), it is an even larger challenge to disentangle excitability changes of the transcranially stimulated neurons from that of transsynaptically stimulated ones within the connected network.

*4.3 Conclusion*

We aimed at disentangling TMS-induced (non-phase-locked) oscillatory activity following $M1_{HAND}$ stimulation from TMS-evoked (phase-locked) responses. Results revealed a specific and dynamic TMS-induced synchronization-desynchronization pattern in the α- and ß-bands, mainly over the stimulated and contralateral sensorimotor cortices that is complementary to TMS-evoked EEG potentials and resting-state EEG. The early α-synchronization was increased by the GABAAergic drugs and decreased by the GABABergic drug, the late α-desynchronization was increased by the GABABergic drug, and the late ß- desynchronization was increased by GABAAergic and GABABergic drugs. These findings are relevant for future investigations of GABA-related alterations in functional brain connectivity, e.g., in brain network disorders such as schizophrenia or epilepsy, as functional connectivity generally employs frequency information as a code.

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